PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **346th Meeting** *of the Biochemical Society was held in St Thomas's Hospital Medical School, London, S.E.* 1 *on* 16*th December,* 1955, *at* 2 *p.m., when the following papers were read:*

COMMUNICATIONS

The Metabolism of 2-Methylnaphthalene. By A. J. GRIMES and L. YOUNG. (*Department of Biochemistry, St Thomas's Hospital Medical School, London, S.E.* 1)

Although studies have been made of the metabolism of toluene and of the di- and tri-methylbenzenes, little is known of the chemical changes which methyl-substituted polycyclic hydrocarbons undergo in the animal body. Special interest attaches to the metabolism of these latter compounds because certain of them show carcinogenic activity. The present report is concerned with the metabolism of 2-methylnaphthalene in the rabbit, rat, guinea pig and mouse.

The compound was administered to rabbits and rats by stomach tube and to guinea pigs and mice by intraperitoneal injection. The urine of the dosed animals contained a variety of metabolites of the hydrocarbon. It was found that, as with toluene, 2-methylnaphthalene is oxidized in the animal body to the corresponding carboxylic acid, for examination of the urine of the dosed animals by paper chromatography showed that 2-naphthoic acid was excreted by the four species studied. This was confirmed in the case of rabbits and rats by the isolation of the acid from the urine. Evidence for the excretion of conjugated 2-naphthoic acid was obtained with all four species and 2-naphthuric acid was isolated from the urine of rats.

2-Methylnaphthalene was found to undergo hydroxylation in all four species, for their urine when examined by paper chromatography showed the presence of 7-methyl-1-naphthol and 7-methyl-2-naphthol, a change analogous to the conversion of naphthalene to 1- and 2-naphthol by these same four species (Corner & Young, 1954). Like naphthalene (Young, 1947), 2-methylnaphthalene was also found to give rise to a dihydrodiol and to a metabolite which yields the original hydrocarbon when decomposed by acid. The structure of the dihydrodiol has been investigated and it is concluded that the compound is 1:2-dihydro-7-methylnaphthalene-1:2-diol. This finding is of interest because no methyl-substituted hydrocarbon has hitherto been shown to give rise to a dihydrodiol in the animal body. The dosed animals of all four species excreted this compound.

It has become apparent, therefore, that whereas the metabolism of 2-methylnaphthalene resembles that of toluene with respect to the oxidation of the methyl group, it resembles naphthalene rather than toluene in giving rise to phenolic derivatives and a dihydrodiol.

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The Lipid (Ether- and Ethanol-extractable Material) of the Livers of Cholesterol-fed Rats. By J. B. M. RATTRAY and R. P. COOK. (*Department of Biochemistry, Queen's College, Dundee, University of St Andrews*)

The livers of rats fed a diet containing 16·6% olive oil and 1·6% cholesterol were dried *in vacuo* and extracted with ether and with ethanol. The total extract was separated by treatment with acetone at varying temperatures into soluble and insoluble fractions which were further separated by adsorption chromatography on silica gel into seven primary fractions. These fractions were further separated by formation of urea adducts and separation by various solvents into final fractions.

The general composition of the main final fractions and yields (as percentage total extract) were: hydrocarbons and higher alcohols forming urea adducts, 2; cholesteryl esters of saturated fatty acids, 2; cholesteryl esters of fatty acids, 24—this fraction contains also free cholesterol and cholestanol; free fatty acids forming urea adduct, 1; glycerides of saturated fatty acids, 19, and of unsaturated fatty acids, 20; phospolipins, etc., 20; water-soluble components, 11. Evidence for the nature of these fractions will be given.

Minor fractions containing 'fast-acting' sterols (0·2%) and fractions with absorption maxima indicative of dienes and dienones (cf. Cain & Morton, 1955) were obtained.

This work was aided by an expenses grant from the Scottish Hospitals Research Endowment Trust.

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Antimycin A in the Study of Succinic Oxidase in Heart-muscle Preparations Deficient in Inorganic Phosphate. By M. B. THORN. (*Department of Biochemistry, St Thomas's Hospital Medical School, London, S.E.* 1)

Experiments previously reported (Thorn, 1955) suggested that the antimycin-inhibitable factor in Keilin & Hartree heart-muscle preparations is freely available to 'succinic dehydrogenase'. This being a property of some interest in a structurally bound system, an attempt has been made to plot the succinic oxidase activity of a given quantity of heart-muscle preparation against increasing concentrations of antimycin-inhibitable factor. Since antimycin A appears to combine stoicheiometrically with the factor (Chance, 1952; Potter & Reif, 1952) it seemed justifiable to use a decreasing scale of μg. antimycin A present per mg. of heart-muscle preparation as an indirect measure of increasing concentrations of factor. From collected data of many experiments a complex curve was obtained, approaching sigmoid form, consisting of a straight line leading into a hyperbola. The hyperbolic portion can be transformed into a straight line by the methods of Lineweaver & Burk (1934). It is unlikely that a complex curve of this character would result from the presence in heart-muscle preparations of a substance competing with the factor for antimycin A. Rather, the curve suggests that, provided sufficient uninhibited factor is present (about 25%), the factor behaves as a coenzyme relatively freely available as hydrogen (electron) acceptor or donator. The maximum velocity was calculated from the Lineweaver–Burk plot, being about 6% greater than the activity of uninhibited succinic oxidase, and also a quantity corresponding to the Michaelis constant, which although of doubtful absolute significance, may be regarded as an inverse measure of the 'affinity' or 'accessibility' of the factor to an adjacent component of the system.

Histidine (Bonner, 1954) and cytochrome *c*, added either separately or together to preparations in media deficient in inorganic phosphate, simultaneously increased the succinic oxidase activity of a control sample and decreased the percentage inhibition of a partially antimycin-inhibited sample. This observation suggests that the factor lies in that part of the succinic oxidase system in which the 'mutual accessibility' (Keilin & Hartree, 1949) of the components is impaired in the near absence of inorganic phosphate. The results of using antimycin A in determining 'maximum velocities' and 'Michaelis constants', as described above, for preparations in low-phosphate media, will be discussed.

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The Effects of Salicylate on Glycogen Content and Oxygen Uptake of the Isolated Rat Diaphragm. By M. J. H. SMITH and SHIRLEY W. JEFFREY. (*Department of Chemical Pathology, King's College Hospital Medical School, London, S.E.* 5)

The injection of salicylate into normal rats produces a marked depletion of liver and muscle glycogen (Lutwak-Mann, 1942; Winters & Morrill, 1955) and an increased oxygen uptake (Meade, 1954). Salicylate has also been reported to cause a number of *in vitro* effects such as diminished glycogen levels in rat-liver slices (Smith, 1955) and increased oxygen uptake in liver and brain preparations (Sproull, 1954; Fishgold, Field & Hall, 1951). The present work is concerned with a study of the *in vitro* effects of salicylate on the glycogen content and oxygen uptake of the isolated rat diaphragm.

Under aerobic conditions, whether a net synthesis or net breakdown of glycogen occurred in the control diaphragms, 5×10^{-3} M salicylate caused a rapid disappearance of glycogen accompanied by an increased production of lactic acid. These effects were reduced at salicylate concentrations of 1×10^{-3} and 5×10^{-4} M and were absent at a concentration of 1×10^{-4} M.

In the presence of 5×10^{-3} M salicylate, the rate of oxygen uptake always showed a marked increase for the initial 30–60 min. of the experiment and then gradually declined, whereas variable effects were obtained with lower concentrations of salicylate.

Under anaerobic conditions 5×10^{-3} M salicylate caused a decrease in glycogen content and an increased lactic acid production. The anaerobic data indicate that the effect of salicylate on glycogen breakdown is not dependent on an increased oxygen uptake although the two effects may be related.

We wish to thank the Diabetic Association for a grant from the Lund Research Fund.

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The Immunological Properties of a Purified Human Fibrinogen. By E. A. CASPARY. (*The Lister Institute, London, S.W.* 1)

An antiserum was obtained by immunizing rabbits intravenously with purified human fibrinogen (Kekwick, Mackay, Nance & Record, 1955) showing only one component in the ultracentrifuge and on electrophoresis, but slightly polydisperse by boundary-spreading methods. The clottable nitrogen of the preparation was 97%; contamination with plasminogen or plasmin was negligible.

To eliminate the effect of thrombin when using the antiserum, the animals were bled into trisodium citrate (1/10 vol. 3·8%) and the plasma defibrinated by the addition of a minimal amount of purified thrombin which was subsequently neutralized by the natural antithrombin. Precipitin tests were made in a citrate–saline solution. The titre after two courses of immunization was 1:32 at 37° with 0·1% (w/v) fibrinogen. In tests keeping the antibody constant, free fibrinogen was detected in the antigen excess region of the antigen–antibody reaction with thrombin, and free antibody in the antibody excess region by titrating with fibrinogen. When tests with plasma fractions were made, a strong reaction was obtained with β-globulin, weak with γ-globulin and no visible effect with albumin and α-globulin. Antiserum absorbed with β-globulin gave a narrower precipitin zone and a fourfold drop in titre. Fibrinogen digested with plasmin to complete loss of clottability reacted with the antiserum with a narrower zone of precipitation; antiserum absorbed with this material failed to react with fibrinogen.

Diffusion in agar (Ouchterlony, 1949) with several fibrinogen preparations showed one major component and one or more faint lines at high antigen concentration. It was evident that some of these lines were caused by α-, β- and γ-globulins.

Plasmin-digested fibrinogen diffuses at a greater rate than fibrinogen and immunological identity cannot be established by this method. An experiment with bovine fibrinogen gave a weak cross-reaction with the antiserum.

One major and two minor components were demonstrated by electrophoresis in agar (Grabar & Williams, 1953) and antibodies to many plasma proteins were detected in the antiserum. Anti-globulin activity was proved by the 'Anti Human Globulin Test' (Coombs, Mourant & Race, 1945).

Thrombin acts on fibrinogen in the presence of the antiserum to give a gelatinous precipitate; the antiserum has no effect on plasma prothrombin conversion.

Immunization of rabbits with physicochemically 'pure' human fibrinogen induced an antibody response which indicates trace contamination with several plasma proteins. The product obtained by digesting fibrinogen with plasmin retained the immunological properties of fibrinogen.

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The Mechanism of Growth of Starch Granules in the Potato Tuber, Studied with Carbon-14. By N. P. BADENHUIZEN and R. W. DUTTON. (*Postgraduate Medical School, Ducane Road, London, W.* 12)

The classical work of A. Meyer (1895) indicated that the growth of starch granules takes place by apposition. The theory implies structural equality of the layers, as has in fact been found (Badenhuizen, 1955). The process could be one of rapid rhythmic crystallization, or one of slow deposition of starch. The alternative theory of intussusception requires the assumption of an outer membrane with special properties. The problem can only be solved by following growth directly. The stripping film technique of autoradiography opens up this possibility.

Using an apparatus developed by one of us (Dutton, 1955) potato plants were allowed to photosynthesize in an atmosphere containing $^{14}CO_2$. Initial CO_2 concentrations from 0·1 to 1·5% were used and activities from 75 to 400 μc incorporated. Activity measurements on various parts of the plant surface indicated a rapid decrease of activity in the leaves and a downward transport to the tubers of active material, which was shown by paper chromatography of extracts to be mainly sucrose. Slices were cut from the tubers and examined by autoradiography. Even after 8 days tubers on the same plant showed a wide range of activities, some being highly active, whilst some were inactive, and others intermediate. Starch was then isolated from the tubers and the granules were examined by the stripping film technique of Pelc (1947).

Native granules showed greater activity at the distal end and at the periphery. After breaking the granules, or gelatinizing them in the presence of saliva, the exposed inner mass was found to be inactive. Corrosion of the granule surface by saliva removed activity, and this process started at the proximal end. Activity was therefore located in a newly deposited layer coating the granule. It was further found that the cytoplasm became active first and then, very gradually, the starch granules. There were also indications that in an intermediate stage amyloplasts became highly active.

We conclude that growth takes place by apposition, and that it may be a very slow process which is entirely dependent on the supply of carbohydrate.

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Anti-metabolites from Vitamin B_{12}. By E. Lester Smith, L. F. J. Parker and Dorothy E. Gant. (*Glaxo Laboratories Limited, Greenford, Middlesex*)

The mono-, di- and tri-carboxylic acids resulting from mild acid hydrolysis of vitamin B_{12} can all be reconverted into the vitamin by reaction in anhydrous dimethylformamide solution first with ethyl chloroformate and triethylamine and then with ammonia (Armitage *et al.* 1953). It was to be expected that treatment of the mixed anhydrides with an amine instead of ammonia would yield the corresponding amides, and that some of these might have anti-vitamin activity.

The reaction mixtures were purified by electrophoresis on paper, to remove unreacted acids and some side-reaction products. The red neutral zones were further purified by repeated paper chromatography with wet *sec.*-butanol containing traces of acetic and hydrocyanic acids. When amino acids or diamines were used, the products were acidic or basic, respectively, and appeared in the appropriate electrophoresis zones.

Crystalline products resulted from the monobasic acids (mixture of isomers or, in a few instances, single isomers) and the 8 amino compounds marked with an asterisk in the list below; other amides did not crystallize. Rigorous purification of the acids used, and of the products, was necessary to remove traces of vitamin B_{12} before anti-vitamin activity could be demonstrated by plate assays with the B_{12}-requiring *Escherichia coli* mutant (see next abstract).

Anti-B_{12} activity was shown by mono-amides of the following: methylamine*, ethylamine*, monoethanolamine, ethylenediamine, dimethylamine, diethylamine*, piperidine*, phenylethylamine, cyclohexylamine, aniline* and sulphanilic acid. Of these, the methylamide appeared the most active, with an inhibition index of about 150. Some larger amines, amylamine*, octylamine and β-naphthylamine gave inactive compounds as did the amino-acids glycine* and alanine and *p*-aminobenzoic acid*. The di-anilides of the dibasic acids were inactive: the tribasic acid gave a weakly active triethylamide and an inactive and almost insoluble tri-anilide. The ethylamides of the monobasic acids without nucleotide were active.

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The Anti-vitamin B_{12} Activity of Some Compounds related to Cobalamin. By W. F. J. CUTHBERTSON, J. GREGORY, P. O'SULLIVAN and H. F. PEGLER. (*Glaxo Laboratories Limited, Greenford, Middlesex*)

Methods have been devised for detecting and assessing compounds that may antagonize the utilization of cyanocobalamin by vitamin B_{12}-dependent organisms.

Qualitative tests were carried out in agar media with *Escherichia coli* (Harrison, Lees & Wood, 1951) and sometimes with *Lactobacillus leichmannii* (Skeggs *et al.* 1950) as well. Into cups cut about 8 mm. apart in vitamin B_{12} assay plates, seeded with the appropriate organism, 3 drops of solution (0·1 μg./ml.) were placed—cyanocobalamin in one cup and test solution (1–1000 μg./ml.) in the other. After overnight incubation the zones of growth were noted. Inert substances did not modify the circular zones of growth around the cups containing the vitamin. Non-specific inhibitors, e.g. phenol, produced clearly defined circular zones of inhibition, cutting sharp circular arcs from the growth zones produced by the vitamin in adjacent cups. Competitive antagonists were expected to depress growth most at low and least at high vitamin concentrations. The inhibition due to such substances should therefore rapidly decrease towards the centre of the growth zones round the adjacent cups and extend further round the edges than when non-specific growth inhibitors are tested. Such effects were in fact found.

The cup-plate method is only qualitative; quantitative assessment of the inhibition index (Shive, 1950) was carried out in fluid media (Harrison *et al.* 1951; Burkholder, 1951; Skeggs *et al.* 1950) by use of graded levels of both vitamin B_{12} and the antagonist. Of the compounds tested the methylamide of the mixed monobasic acids derived from cobalamin (Smith, Parker & Gant, 1955) was the most active (inhibition index of 150 with *E. coli*); the anilide of acid E 2 and the diethylamide of the mixed monobasic acids were less active (inhibition indices 2000–3000 with *E. coli*).

Vitamin B_{12}-deficient rats were given varied levels of vitamin and antagonist. In the individual tests the antagonists depressed growth, though not significantly, but when all results on the different antagonists were combined a significant growth depression ($0{\cdot}05 > P > 0{\cdot}001$) could be demonstrated at doses of 0·1–3 μg./day. The data are limited owing to the small amounts of material available and are not sufficient to establish whether or not these effects can be reversed by cyanocobalamin. Attempts were made to detect substances inhibiting vitamin B_{12} synthesis but no positive results were obtained.

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The Estimation of Oestrone, Oestradiol-17β, and Oestriol in Human Urine by Gradient Elution Partition Chromatography. By ELSIE H. AITKEN and J. R. K. PREEDY (introduced by C. J. O. R. MORRIS). (*Medical Unit, London Hospital, E.* 1)

Twenty-four hour urine aliquots (about 3 % of total volume) were extracted by the procedure of Engel *et al.* (1950), except that autoclaving was replaced by refluxing for 0·5 hr. in 1·8 N-H_2SO_4. The dried extract was transferred quantitatively to a partition column, 15 cm. long, 0·57 cm. in internal diameter, packed with Hyflo Super-Cel as supporting phase. The Super-Cel had previously been mixed with stationary phase (70 vol. CH_3OH—30 vol. distilled water) in the proportion 0·66 ml./g. Super-Cel. Chromatography was begun with a mobile phase containing 32 % (v/v) CCl_4—68 % petroleum ether (boiling range 60–80°). Fifteen 1 ml. fractions were collected. The mobile phase was then changed to 23 % (v/v) CCl_4—12 % $CHCl_3$—65 % petroleum ether. A further fifteen 1 ml. fractions were collected. The mobile phase was then again changed to 45 % $CHCl_3$—55 % petroleum ether. A further twenty-four 1 ml. fractions were collected. The dried fractions were estimated for oestrogen content by fluorimetry (Engel *et al.* 1950). Automatic changes of mobile phase were accomplished by attaching to the column a U-shaped glass reservoir, the two limbs of which, containing 32 % CCl_4—68 % petroleum ether and 45 % $CHCl_3$—55 % petroleum ether respectively, were constructed of tubing of the necessary lengths and internal diameters to obtain successively the volumes and compositions of mobile phase required. This chromatographic procedure enabled the three oestrogens to be separated not only from one another, but also from

the large amounts of fluorescent interfering material present in the urine extracts, by means of a single partition column allowed to run overnight. Preliminary recoveries of pure crystalline oestrone, oestradiol-17β and oestriol, 0·2–1·0 μg. of each added to male and female urine aliquots, were as follows (means and ranges): oestrone 86% (81–91), oestradiol-17β 88% (83–94), oestriol 79% (70–85). Normal urine oestrogen outputs so far obtained were (μg./day): male, oestrone 2·4–4·0, oestradiol-17β 2·0, oestriol $<$ 2·0; female, oestrone 2·9–5·1, oestradiol-17β $<$ 2·0; oestriol 2·9–7·3.

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Inhibition of Dihydrocozymase Oxidase Activity of Pig-Heart Muscle and Bacterial Preparations by 2-Heptyl-4-hydroxyquinoline-*N*-oxide. By F. L. JACKSON* (*King's College Hospital Medical School, London*) and J. W. LIGHTBOWN (introduced by J. H. HUMPHREY) (*National Institute for Medical Research, Mill Hill, London*)

The Keilin and Hartree type of heart muscle succinic oxidase preparation will also oxidize dihydrocozymase (DPNH), and this process can be followed by measuring the decrease in absorption at 340 mμ., as described by Slater (1950). Using this method, we have been able to demonstrate inhibition of DPNH oxidation when 2-heptyl-4-hydroxyquinoline-*N*-oxide (heptyl-*N*-oxide) was added to the preparation. Concentrations of 1·5 μg. of heptyl-*N*-oxide/ml. produced 90% inhibition of activity. Similar experiments with cell-free preparations of *Staphylococcus aureus* showed approximately the same degree of inhibition.

With the heart-muscle preparation, the anaerobic Thunberg technique with methylene blue was used to investigate possible effects on diaphorase activity. Concentrations of heptyl-*N*-oxide of 5 μg./ml. did not increase the methylene-blue reduction time.

In heart muscle succinic oxidase system, the site of action appears to be between succinic dehydrogenase and cytochrome *c* (Lightbown & Jackson, 1954). Slater (1950), who excludes cytochrome *b* from the DPNH oxidase system, has postulated that diaphorase is linked to cytochrome *c* through an intermediate factor, the same factor linking cytochromes *b* and *c* in the succinic oxidase system. If this is so, the factor, which has been suggested by Potter & Reif (1952, 1954) as the site of action of antimycin A, might also be the site of action of the heptyl-*N*-oxide. However, we have observed spectroscopically that cytochrome *b* is rapidly and strongly reduced by DPNH in the presence of either heptyl-*N*-oxide or antimycin A, and that cytochromes *c* and *a* are only slowly reduced under these conditions.

Since the inhibitors interfere with electron transport between cytochromes *b* and *c*, strong reduction of cytochrome *b* by DPNH would suggest that cytochrome *b* is on the pathway for DPNH oxidation. While their site of action might be on a factor between cytochromes *b* and *c*, it is possible that the inhibitors might react with cytochrome *b* itself, so as to prevent its oxidation by way of the cytochrome *c*–cytochrome oxidase pathway, but not its reduction by succinic dehydrogenase or diaphorase.

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Analysis of Steroids in Adrenal Venous Blood and in Urine in a Case of Virilism. By I. E. BUSH (*St Mary's Hospital, London, W.* 2), J. SWALE and J. PATTERSON (*Charing Cross Hospital, London, W.C.* 2)

The work of Callow first demonstrated the importance of dehydro*iso*androsterone (DHIA) as a human metabolite (Callow & Callow, 1939). This substance is the preponderant 17-ketosteroid in most normal people (Lieberman & Teich, 1953). There is strong evidence that DHIA is derived from the adrenal cortex, but Lieberman & Teich (1953) suggest that it is not secreted by the adrenal as such, but results from a precursor. We have obtained evidence that this suggestion is largely correct.

Adrenal venous blood was obtained at operation for adrenalectomy on a case of post-pubertal virilism; 24 hr. urine collections were made on this

day and during her stay in hospital. ACTH (gel 2 × 50 U.) was administered during the day of operation to ensure maximal secretion rates. 30 ml. blood was collected in 15 min. from the left adrenal vein and analysed by paper chromatography (Bush, 1952, 1954) after extraction in the usual way. The sample contained approximately (±20%) 67 μg. hydrocortisone (F), 67 μg. corticosterone, 28 μg. 11-β-hydroxyandrost-4-ene-3:17-dione, 28 μg. DHIA, 12 μg. androsterone. Urinary excretion (mg./24 hr.) for that day was: tetrahydrocortisone, 2·90; tetrahydroF, 1·2; androsterone, 4·35; etiocholanolone, 2·70; DHIA, 5·6; 11-β-hydroxyetiocholanolone, 0·17; 11-ketoetiocholanolone, 0·45; 11-β-hydroxyandrosterone, 0·74.

No peripheral blood was obtained, but it is unlikely that more than 5% of each compound in the blood sample arose from arterial blood (Bush & Sandberg, 1953; Migeon & Plager, 1954).

Back-calculation from the urinary figures using percentage conversion values similar to Dorfman's (1954) for 11-oxygenated 17-ketosteroids and reducing steroids gives a calculated hydrocortisone/11-β-hydroxyandrost-4-ene-3:17-dione secretory ratio of 2·2–3·35 (observed 2·4). The observed hydrocortisone/DHIA ratio (2·4) however would require an excretion of 82–88% unchanged DHIA to account for urinary DHIA. Even allowing for earlier methods, this is an unreasonably high figure (Mason & Kepler, 1946). If a 5% excretion is assumed for DHIA, 4·25% of urinary DHIA would be accounted for by secreted DHIA. This suggests strongly that >90% of urinary DHIA was derived from an unidentified adrenal precursor in our case.

Dorfman's (1954) concepts of the origin of 11-oxygenated 17-ketosteroids receive considerable support from this case in view of the agreement between calculated and observed hydrocortisone/11-β-hydroxyandrost-4-ene-3:17-dione ratios.

We are indebted to Mr L. R. Broster for obtaining this crucial blood sample.

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Nucleotide Sequences in Yeast Ribonucleic Acids. By W. E. COHN and R. MARKHAM. (*University Chemical Laboratory, and Agricultural Research Council Virus Research Unit, Molteno Institute, Cambridge*)

Information as to the variety of sequences of nucleotides existing in ribonucleic acids is dependent upon the isolation and characterization of polynucleotides obtained from them. Exhaustive degradation by pancreatic ribonuclease yields mixtures of polynucleotides containing all those sequences which are resistant to the enzyme. Of the sixteen possible dinucleotide combinations, linked 3′ to 5′ and possessing a free 3′-phosphate, four are found as dinucleotides in ribonuclease digests (AC, AU, GC, GU; A, G, C and U represent adenylic, guanylic, cytidylic and uridylic acids respectively and the 3′–5′ internucleotide link runs from left to right), and four others exist as portions of trinucleotides and larger polynucleotides (AA, AG, GA, GG) (Markham & Smith, 1952; Volkin & Cohn, 1953). The remaining eight are not found in exhaustive ribonuclease digests, due to the specificity of the enzyme, and its transferase activity reduces the significance attached to the finding of such sequences in partial digests. Brief acid hydrolysis, however, has indicated that yeast ribonucleic acids contain the sequences CC, CA and UC and/or CU (Merrifield & Woolley, 1952). This type of hydrolysis was employed by us to find the missing sequences and to determine the relative amounts of isomeric pairs (e.g. GC and CG).

Commercial yeast ribonucleic acid, reprecipitated, was treated for 2·5 min. with 6N-HCl at 20°, neutralized, and separated into known polynucleotide fractions by paper chromatography. From these fractions, pairs of isomeric dinucleotides (e.g. GC and CG) having identical electrophoretic mobilities were isolated by paper electrophoresis, eluted, treated with ribonuclease and rechromatographed. The mononucleotides liberated were identified by chromatography and by spectrum. The unhydrolysed dinucleotide was then hydrolysed and chromatographed to determine its structure.

The results indicate that those sequences not previously found are present in yeast ribonucleic acids in amounts not grossly differing from those of

the other sequences. Thus it would appear that all sequences of dinucleotides are to be found in yeast ribonucleic acids.

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Distribution of Pressor Amines and Adenosinetriphosphate (ATP) in Adrenal Medullary Granules. By H. Blaschko, G. V. R. Born, A. D'Iorio and N. R. Eade. (*Department of Pharmacology and Nuffield Institute for Medical Research, University of Oxford*)

Homogenates of the bovine adrenal medulla are known to contain the bulk of their adrenaline and noradrenaline in granules with sedimentation properties similar to those of mitochondria; in these granules the amines are mainly present in a condition in which they are not able immediately to exert their biological action. The 'mitochondrial' fraction can be subdivided into a 'bottom' layer of granules with a relatively high amine content and a 'top' layer less rich in amines (Blaschko, Hagen & Welch, 1955).

Högberg, Hillarp & Nilsson (1955) have recently reported the occurrence of large amounts of ATP in adrenal medullary granules. We have therefore studied the distribution of ATP in fractions obtained from the bovine adrenal medulla, in relation to their content of catechol amines. ATP was determined by the firefly luminiscence test (Born & Bülbring, 1955). It was found that the bottom layer was extremely rich in high-energy phosphate; in three experiments the concentrations of ATP, on the basis of dry weight, were 7·7, 7·9 and 4·5% respectively; in the same experiments the top layer, which is less rich in catechol amines, contained 0·9, 1·2 and 2·0% of ATP.

Upon transfer into hypotonic media the granules are known rapidly to release the catechol amines; this treatment was also found to release the ATP. Resuspension of the bottom fraction in 0·2 M sucrose released about one-half of both ATP and catechol amines; in 0·1 M sucrose practically all the ATP and catechol amines were found in the supernatant. No evidence for destruction of ATP was obtained in these experiments.

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Studies on the Lipolytic System Induced in Plasma by Heparin Injection. By D. S. Robinson. (*Sir William Dunn School of Pathology, University of Oxford*)

Hahn (1943) observed that the intravenous injection of heparin abolished the alimentary lipaemia which is normally present during fat absorption. Subsequent studies on this 'clearing system', induced in plasma by heparin injection, have shown that it represents a lipolytic process in which the triglyceride of the lipid particles is hydrolysed to free fatty acid with the formation of a soluble fatty acid-albumin complex in the plasma (Robinson & French, 1953). The addition of heparin to plasma *in vitro* does not produce an active 'clearing system'.

In the experiments to be reported active plasma, obtained from rats injected with heparin, has been passed through a column of 'De-Acidite F' (Permutit Co. Ltd.), a strongly basic anion exchange resin, at pH 7·4 and 0°. Such treatment results in the removal of heparin from the plasma and the heparin can be recovered by appropriate treatment of the column.

The 'clearing activity' of the effluent from the column has been studied using the techniques already developed in this laboratory. It appears that while the removal of heparin from the plasma does not destroy the enzyme system it does markedly reduce its stability. Thus, after only 10 min. incubation of the effluent at pH 7·4 and 37°, the clearing activity falls to 10% of its initial value. If small quantities of free heparin are added to the effluent prior to its incubation the

enzyme system regains the stability of the original plasma and no detectable fall in activity occurs during incubation for 10 min. at 37°.

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The Effect of Hexoestrol on Cholesterol Metabolism in the Rat. By G. S. Boyd and W. B. McGuire. (*Department of Biochemistry, University of Edinburgh*)

Studies have been made of the effect of the synthetic oestrogen hexoestrol on cholesterol metabolism in the young male rat. Since hexoestrol causes lipid depletion of the adrenal cortex (Vogt, 1945) and apparently induces adrenal insufficiency (Vogt, 1955), metabolic effects of this substance were studied in both intact and adrenalectomized 5–7 week old rats. Rats were fed *ad lib.* during the experiments. Cholesterol was estimated by a micro-modification of the Sperry & Webb method (1950) and total liver glycogen by the anthrone method of van der Vies (1954).

Intact rats receiving 70 μg. hexoestrol/100 g. body weight/day for 6 days progressively lost weight and a marked serum hypocholesterolaemia developed from a control level of 54, to 23 mg. cholesterol/100 ml. serum. Adrenal cholesterol decreased from a control level of 1·26 to 0·820 g./100 g. tissue. Liver slices from control animals and from hexoestrol-treated animals were incubated for 3 hr. at 37° under 100% oxygen in Krebs phosphate solution (Krebs & Henseleit, 1932) in the presence of 3 μM sodium [1-^{14}C]-acetate and the percentage incorporation of acetate into cholesterol determined. It was found that hepatic cholesterol biosynthesis was depressed to 51·7% of the control value. Administration of 70 μg. hexoestrol/100 g. body weight/day for 13 days intensified the effects mentioned above, reducing the serum cholesterol from 73 to 23 mg. cholesterol/100 ml. serum, the adrenal cholesterol from 2·87 to 0·515 g./100 g. tissue and hepatic cholesterol biosynthesis to 11·1% of the control value; liver cholesterol fell from 234 to 161 mg./100 g. tissue and liver glycogen from 9·21 to 5·61 g./100 g. tissue. Pituitaries and adrenals were hypertrophied and the haematocrit value remained at the control level throughout the experiment.

The administration of 50 μg. hexoestrol/100 g. body weight/day for 7 days to adrenalectomized rats resulted in a very marked serum hypocholesterolaemia from 42 mg. cholesterol/100 ml. serum in adrenalectomized control rats to 6 mg. cholesterol/100 ml. serum in hexoestrol treated adrenalectomized rats. Thus hexoestrol appears to inhibit cholesterol synthesis in the male rat and this inhibition may be independent of the adrenal gland.

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